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Toxicology of Chemical Mixtures: A Review of Mixtures Assessment

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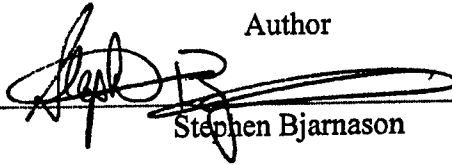
Toxicology of Chemical Mixtures: A Review of Mixtures Assessment.

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Abstract

The science of risk assessment revolves broadly around hazard identification (toxicity) and exposure assessment information. While exposure to environmental hazards most often occurs with complex chemical mixtures, the majority of existing toxicity data is for single compounds or simple mixtures, thus presenting problems to the risk assessor. Several approaches to assess mixtures have been developed (e.g. Hazard Index; Target-organ Toxicity Dose; Weight-of Evidence; Toxic Equivalence), each of which have their limitations, primarily with respect to the prediction of potentially unforeseen interactions between the mixture constituents which may affect their resultant toxic outcome. Recent advances in disciplines such as genomics, proteomics, metabonomics and physiologically-based pharmacokinetic modeling should assist in the hazard assessment of complex chemical mixtures. However, the process of regulatory assessment of these types of exposures will remain both complex and difficult.

Résumé

La science de l'évaluation des risques consiste principalement à identifier les dangers (toxicité) et à évaluer l'information liée à une exposition. L'exposition aux dangers environnementaux consiste souvent en des mixtures chimiques complexes mais la majorité des données existantes de toxicité consiste en un seul composé ou en des mixtures simples, causant par conséquent, des problèmes à l'évaluateur des risques. Plusieurs méthodes évaluant les mixtures ont été développées (par ex. : Indice des risques, Dose de toxicité de l'organe cible, Valeur probante; Équivalence toxique) chacune comportant des limites ayant surtout à voir avec la prédiction d'interactions imprévues potentielles entre les composants de la mixture qui peuvent affecter les résultats toxiques. Les progrès récents dans des disciplines telles que la génomique, la protéomique, la métabonomique et la modélisation pharmacocinétique à base physiologique devraient faciliter l'évaluation des dangers dans les mixtures chimiques complexes. La procédure d'évaluation des règlements de ces types d'exposition demeure cependant à la fois complexe et difficile.

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Executive summary

Exposure to environmental contaminants occurs to mixtures and not to single contaminants. These exposures may also occur across multimedia environments, soil, water and air, which significantly complicates an assessment of impact on health. The problems of assessing environmental contaminants are two fold; understanding the potential effects and determining exposure concentration. The toxicology of complex mixtures is very poorly understood and the capture of relevant exposure information for mixtures has been a problem. This paper focuses on chemical hazards and how different agencies assess mixtures in occupational and environmental settings. Both exposure scenarios were examined. Troops on deployment may be faced with occupational levels of exposure to mixtures, but the length of exposure may be closer to that evaluated for an environmental setting (i.e. 24 hours per day, 7 days per week).

Very few mixtures have been studied as complete mixtures in a laboratory setting, therefore several evaluation approaches have been developed. These methods are based on toxicological information that is known for the constituent compounds, as well as available exposure information. Each approach makes certain assumptions to reach a conclusion and these are often a limiting factor in the utility of the approach. These approaches are used by governmental and non-governmental agencies around the world for occupational and environmental assessments of mixtures.

Occupational exposure assessment generally assumes a higher dose or exposure level than an ambient or environmental assessment because the former is generally for a specified time period (e.g. 8 hours/day; 5 days/week) in a very specific environment. Occupational health organizations have developed evaluation systems for both non-cancer causing and cancer causing mixtures using different assessment schemes based on the level of available information. Agencies evaluating environmental exposures have developed similar assessment tools, but the exposure levels are lower and the time period is continuous (i.e. 7 days per week). As with occupational assessments, different schemes are used for cancer and non-cancer causing components of complex mixtures.

Assessment of complex chemical mixtures is hampered by the lack of toxicity data on complete mixtures, as well as the potential for chemical interactions within the mixture that may result in unforeseen by-products that have an inherent toxicity. Another factor that is not generally accounted for is the effect of stress on exposure and resultant toxicity, and also of exposure-induced stress.

There is significant effort being applied to develop an understanding of how complex mixtures should be assessed and these efforts are being led by civilian agencies and organizations. The information, guidelines and regulations coming from these efforts can act as a guide to assist in developing methodologies to assess risk to troops in different chemical environments.

This report was written to fulfill a commitment to NATO TG-009 (Protection Against Adverse Effects of Toxic Hazards). A presentation based on the report was made to this group in Delft, The Netherlands. 19 February 2004.

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Sommaire

L'exposition aux contaminants environnementaux existe au contact de mixtures et non d'un seul contaminant. Ces expositions peuvent aussi exister dans des milieux multiples, des sols, l'eau et l'air ce qui complique l'évaluation de leur impact sur la santé. Le problème de l'évaluation des contaminants environnementaux présente deux aspects : la compréhension des effets potentiels et la détermination de la concentration de l'exposition. La toxicologie des mixtures complexes est très peu comprise et la sélection de l'information pertinente concernant les mixtures a toujours posé un problème. Cet article se concentre sur les dangers chimiques et sur la façon dont les agences évaluent les mixtures dans des contextes professionnels et environnementaux. Les deux scénarios d'exposition ont été examinés. Les troupes déployées peuvent avoir à faire face à l'exposition aux mixtures dans le contexte de leur profession mais la durée de l'exposition peut être plus proche de celle évaluée pour des contextes environnementaux (par ex. : 24 h par jour, 7 jours par semaine).

Très peu de mixtures ont été étudiées dans leur totalité en laboratoire, par conséquent plusieurs méthodes d'évaluation ont été développées. Ces méthodes sont basées sur l'information toxicologique connue pour les composés ainsi que sur l'information disponible concernant l'exposition. Chaque méthode atteint une conclusion à partir de certaines assumptions ce qui est souvent un facteur limitatif quant à l'utilité de la méthode. Ces méthodes sont utilisées par les agences gouvernementales et non gouvernementales du monde entier en ce qui concerne l'évaluation de ces mixtures dans le contexte professionnel et environnemental.

L'évaluation de l'exposition dans le contexte professionnel considère, en règle générale, que la dose ou le niveau d'exposition est plus important que pour une évaluation environnementale parce que la première évalue normalement une durée de temps spécifique (par ex. : 8 heures par jour ; 5 jours par semaine) dans un milieu très spécifique. Les organisations de la santé du travail ont développé des systèmes d'évaluation pour des mixtures qui causent le cancer et pour celles qui ne le causent pas, utilisant des stratagèmes d'évaluation différents basés sur le niveau d'information disponible. Les agences évaluant les expositions environnementales ont développé des outils d'évaluation similaires mais les niveaux d'exposition sont plus bas et la durée de temps est continue (par ex. : 7 jours par semaine). Tout comme pour les évaluations dans le contexte d'une profession, des stratagèmes différents ont été utilisés pour les composants de mixtures complexes causant et ne causant pas le cancer.

L'évaluation de mixtures chimiques complexes est retardée par le manque de données concernant la toxicité de la totalité des mixtures ainsi que par le potentiel d'interaction chimique entre la mixture pouvant résulter de sous-produits imprévus qui possèdent une toxicité inhérente. Un autre facteur dont on ne tient normalement pas compte est l'effet du stress sur l'exposition et la toxicité qui en résulte ainsi que du stress induit par l'exposition.

On s'efforce principalement de comprendre comment les mixtures complexes devraient être évaluées et ces efforts sont conduits par des agences et des organisations civiles. L'information, les lignes directrices et les règlements qui résultent de ces efforts servent de guide au développement des méthodologies et à l'évaluation du risque posé aux troupes se trouvant dans des milieux chimiques différents.

Ce rapport a été écrit pour s'acquitter d'un engagement par rapport au groupe technique TG-009 de l'OTAN (Protection contre les effets néfastes des risques toxiques). Une présentation basée sur le rapport a été faite à ce groupe à Delft, aux Pays-Bas, le 19 février 2004.

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Introduction

The science of risk assessment revolves broadly around hazard identification (toxicity) and exposure assessment information. While exposure to environmental hazards most often occurs with complex chemical mixtures, the majority of existing toxicity data is for single compounds or simple mixtures, thus presenting problems to the risk assessor. For decades now, regulators worldwide have grappled with the complexities of risk assessing chemical mixtures [1-3].

Xenobiotic exposures commonly occur across multimedial environments, including water, air and soil, that act to modulate the actual dose received by the exposed individual. Simple everyday activities such as bathing, eating, drinking and moving from an indoor to outdoor environment complicate the exposure assessment to even single toxic compounds. The adequate capture of such exposure data has been an ongoing problem and the development of sensitive and accurate personal dosimetry is an active field of study. Further complicating exposure assessment is the necessity of understanding the environmental fate of the compounds in question: how they interact in and with the environment can alter both the toxicity and exposure scenarios. An example of this is the combination of volatile organic compounds with oxygen and sunlight to produce ground level ozone, a common constituent of urban smogs. Exposure assessment is critical to understanding the risks posed by exposure to hazards in the environment.

Hazard identification is the toxicology (animal and human) and epidemiology that provides the biological information on the response of an organism to the exposure (dose) of a toxic substance. Dose-responses can be threshold, linear non-threshold or hormetic [4-6]. The responses measured can range from acute to chronic toxicity.

This review will focus only on chemical hazards, although "hazard" may be defined as being either chemical, physical (e.g. radiation) or biological in nature. Furthermore, although it is recognized that the risk assessment of militarily relevant toxicants in the field may involve chemical exposures and assumptions that are unique to military science, we will focus on how different agencies assess them in occupational and environmental scenarios; their different approaches being instructive to the science as a whole. This review is not exhaustive in breadth and is only intended to provide an overview of how chemical mixtures toxicology assessment is being pursued.

Different approaches to mixture assessment

Several techniques have been devised to assess the toxicity of chemical mixtures. An excellent review of these different methods can be found in a recent Agency for Toxic Substances and Disease Registry (ATSDR) guidance document [2]. Each of the assessment methods mentioned in the ATSDR document are briefly described below.

One of the simplest ways to assess mixtures is the Hazard Index (HI), which uses dose additivity. For each component of the mixture, the exposure level is divided by a defined level of exposure that causes a toxicological effect (e.g. Threshold Limit Value, TLV). This ratio is calculated for all components of a mixture and summed to define the HI for the mixture. As the HI approaches unity, there may be concern for effects from the mixture. The HI method does not consider interactions between components of the mixture.

A modification of the HI is the Target-organ Toxicity Dose (TTD) method which allows for assessment of chemical mixtures where the components do not all have the same critical toxic effect. If components of a mixture have effects on different systems in the body, the TTD accounts for this when the component level reaches a threshold where the critical effect will occur. The TTD is calculated for each endpoint of concern and then used to estimate endpoint-specific hazard indexes. When any of these HI approach or exceed unity, the potential for toxic effects from exposure to the mixture is increased.

The Weight-of-Evidence Modification to HI (WOE) accounts for interactions by using weight of evidence for interactions among pairs of components of the mixture. Each possible pair of chemicals is evaluated in order to make binary weight-of-evidence determinations for the effect of each chemical on the toxicity of each other chemical. In the WOE modification, changes in proportions of mixture components are not accounted for and the model assumes that all chemical interactions are only binary in nature (A can affect B and B affect A but C does not affect these interactions).

Toxic Equivalency and Relative Potency compares mixture components against a component that has been sufficiently well investigated with respect to health information. This technique assumes dose additivity, and the assessment is expressed as toxic "equivalents" of a known component (usually the most toxic) of the mixture. Environmental samples containing dibenzo(p)dioxins are perhaps the best known examples of being assessed using the toxic equivalency approach; their potential toxicity often being expressed as "2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalents".

Two other methods that may be used for carcinogenic endpoints are the Total Cancer Risk and the Integral Search System (ISS). Total Cancer Risk assumes the response or risk for a mixture is the sum of the risks for cancer for all of the components based on the dose and potency parameters for each component. ISS uses data for binary mixtures to predict the hazard of exposure to mixtures of three or more chemicals. ISS does not include exposure information or dose as part of the assessment.

Physiologically-based pharmacokinetic modelling (PBPK) uses computer modelling of biological information to assess the potential for interactions between chemicals in biological systems. PBPK modelling can be used to predict effects from co-exposure for different scenarios. These scenarios may represent changes in components in the mixture or changes in exposure concentrations.

A variety of different approaches have been utilized by various national and international organizations to assess how chemical mixtures potentially affect human health with respect to occupational and environmental exposures. An examination of these methods, the assumptions upon which they rest and their subsequent shortcomings, should provide insight as to how to approach the hazard assessment of mixtures in a military operational scenario.

Occupational exposure assessment

Occupational exposure assessment generally examines higher dose/exposure levels than an ambient or environmental assessment. This is because the occupational situation generally occurs for a specified time period (e.g. 8 hours/day; 5 days/week) in a specific environment. Different countries and political/economic blocks have developed ways of evaluating occupational exposures to mixtures. Many of these are simply harmonized labelling or classification conventions but they could be used for a cursory assessment of chemical mixtures.

Canada, at the federal level, has established the Workplace Hazardous Materials Information System (WHMIS) which uses the "Controlled Products Regulations" (CPR) legislation to classify chemicals and mixtures based on different endpoint criteria (CPR 46 to 65). For each compound, a WHMIS classification is established. For mixtures, a toxicological evaluation is carried out by taking the LD₅₀ or LC₅₀ of every ingredient present at a concentration of one per cent or more. If this information is known, the LD₅₀ or LC₅₀ of the mixture is determined by calculation of a proportional representation of the constituent compounds. If the LD₅₀ or LC₅₀ of one or more of the ingredients is not known, the LD₅₀ or LC₅₀ of the mixture is equal to the LD₅₀ or LC₅₀ of the most acutely lethal ingredient that is present in the mixture at a concentration of one percent or more and for which LD₅₀ or LC₅₀ data is available. This legislation relies heavily on Test Guidelines from the Organization for Economic Co-operation and Development (OECD) for specific endpoints assessment. See [7] for an overview and links.

In the USA there are a variety of occupational regulatory agencies that vary in their approach to risk assessment. The Occupational Safety and Health Administration (OSHA) sets a permissible exposure limit (PEL) for occupational exposures. OSHA recommends a hazard index approach similar to the American Conference for Governmental Industrial Hygienists (ACGIH; see below) where the sum of the ratios must not exceed unity (i.e., one). The approach is not restricted to substances that have similar effects. OSHA places the responsibility for evaluation on the manufacturer, importer or employer and if a mixture has been tested as a whole, the results are used to determine if the mixture is hazardous. If the mixture has not been tested as a whole, it is assumed that the mixture will present the same health hazards as do the components which comprise one percent (by weight or volume) or greater of the mixture. The exception to this is if a component is a carcinogen which comprises 0.1% or greater of the mixture. In this case, the mixture will be considered as a carcinogenic hazard. The potential physical hazard presented by a mixture must be addressed as well. If a component present in a mixture in concentrations of less than one percent (<0.1% for carcinogens) could be released in concentrations that could exceed an OSHA PEL or ACGIH Threshold Limit Value (TLV), or could present a health risk at those concentrations, the mixture assumes the same hazard as that component. (OSHA Regulations (Standards – 29 CFR) Air contaminants. – 1910.1000; Hazard Communication - 1910.1200). See [8].

The National Institutes for Occupational Safety and Health (NIOSH) in the USA has recognized the need for further development of assessment methodologies with respect to mixture toxicology [9].

The strategies for non-cancer and cancer-causing effects from exposure to mixtures being proposed by the ATSDR in the USA are similar (See Fig 2 and 3; [2]). The executive summary in this document [2] provides a clear explanation on how the ATSDR proposes to assess exposures to chemical mixtures ... "Exposure data and toxicological information on the mixture of concern (or a similar mixture) are the preferred basis for an assessment. If available, toxicological information on mixtures of concern are reviewed and evaluated from ATSDR documents, including interaction profiles and toxicological profiles. If specific ATSDR documents or comparable documents from other agencies are not available, or do not provide Minimal Risk Levels (MRLs) or comparable health guideline values for the mixture or guidance regarding a health assessment approach, and if suitable whole mixture studies are not available, a components-based approach is undertaken. The components-based approach

focuses on mixture components that are present at toxicologically significant exposure levels, based on estimated exposures and relevant health guideline values. Linked physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) models for two or more components, if available, may be used to predict the potential for interactions, or possibly for non-cancer or cancer health effects from the mixture. The hazard index method is used to screen for non-cancer health hazards from potential additivity of the components. Cancer risks for the components are summed to screen for health hazards from potential additivity of carcinogenic effects. A weight-of-evidence method is used to evaluate the potential impact of interactions on non-cancer and cancer health effects." [2]. ATSDR reviews both occupational and environmental exposure.

In the European Union, a committee was established to provide advice on chemical exposure. The Scientific Committee on Occupational Exposure Limits (SCOEL) [10] started as an informal group of scientists formed to provide advice to the European Commission and eventually was formalized in 1995. Experts in chemistry, toxicology, epidemiology, occupational medicine and industrial hygiene are appointed to this group. The SCOEL makes recommendations on health based Occupational Exposure Limits (OEL) which may include eight-hour time weighted averages (TWA), short-term / excursion limits (STEL) and biological limits. The SCOEL evaluates all available data and then proposes a recommendation which provides the scientific basis for exposure limits included in legislation. Member States then utilize this information to establish exposure values. It is not explicitly clear how SCOEL assesses chemical mixtures or if there is a formalized process.

In Australia, the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) [11] and the National Occupational Health and Safety Commission [12] (specifically NOHSC:1008(1999)) are responsible for providing guidance on assessment of chemical mixtures.

Other organizations have also developed, or started to develop, guidelines and recommendations for exposures to chemical mixtures. Perhaps the most germane to this paper is the USA National Academies of Science current project evaluating Toxic Industrial Chemicals and chemical warfare agents [13] (see Review of the Army's Technical Guidance Documents on Assessing Toxicological Risks From Exposures to Chemicals). This is a review of the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 230 entitled "Chemical Exposure Guidelines for Deployed Military Personnel".

The American Conference of Governmental Industrial Hygienists in the USA utilizes an additivity approach for assessing the hazards posed by mixtures (hazard index approach) [14]. The substances must act on the same organ system. The ratio (C/T) of the exposure concentration (C) to the TLV (T) for each substance is summed. If the sum exceeds one, the TLV for the mixture has been exceeded. This approach is not used if the effects are on different systems. If the primary effect of the different components are not additive but are independent, e.g. when different organs are affected, then the TLV is considered to be exceeded when at least one component C/T ratio in the mixture exceeds one. When a number of harmful dusts, fumes, vapours, or gases are released, ACGIH indicates that the only feasible approach may be to measure a single substance in order to evaluate the hazard. The threshold limit for this substance should be reduced by some factor (the magnitude of which takes into account the number, toxicity, and relative amounts of the other components typically present).

The United Nations Economic Commission for Europe (UNECE) has set forth to establish a globally harmonised system (GHS) of hazard classification and labelling for the safe use of chemicals in the workplace and the environment in general [15]. The hazard classification process refers principally to the hazards arising from the intrinsic properties of chemical elements and compounds and mixtures of these elements (Part 2 and 3 [15]). Substances are classified according to their health, environmental and physical hazards. Mixtures are handled in the following manner: a) classification of the mixture will be based on test data for the complete mixture, if available, or b) bridging principles (see Section 3.1.3.5 in Part 3 [15]) will be used to determine classification of the mixture. For health and environmental classification, if neither "a" or "b" are sufficient then a method is agreed upon based on information known to classify the mixture. Specific considerations for classifying mixtures have been identified but a significant level of responsibility rests with the reviewer/evaluator in the final classification.

The Organization for Economic Co-operation and Development established an advisory group to examine harmonisation of classification and labelling of compounds and this group in turn, established an expert group to study mixtures. This program does not require testing of chemicals but makes use of existing data. The criteria are laid out in detail in [16]. Briefly, the process of classification is as follows: a) where data are available for the complete mixture, classification will be based on that data; b) where data are not available for the complete mixture, bridging principles are to be considered (see [16] for detailed explanation of principles); c) if information is not sufficient to allow for bridging principles to be applied, then agreed methods for estimating the individual hazards based on known information will be applied. Part 3 of the OECD monograph [16] provides details on the classification of chemical mixtures which cause acute toxicity, skin and eye corrosion/irritation, respiratory or skin sensitisation, germ cell mutagenicity, carcinogenicity, reproductive toxicity, or specific target organ systemic toxicity.

Environmental exposure assessment

Ambient or environmental exposure to pollutants means non-occupational exposures. These are generally at lower exposure concentrations for longer periods of time (e.g. 24 hours per day for 7 days per week). They encompass indoor and outdoor environments and may even include microenvironments such as inside a vehicle.

In Canada, environmental exposure assessment to toxics are done under the Canadian Environmental Protection Act (CEPA). With respect to mixtures, the approach as to whether a chemical substance is toxic under CEPA is dependent upon the nature of available data on the mixture. As with occupational exposures, at times the mixture composition, exposure levels and toxic effects may be well characterized. Generally, this is not so and the assessment is usually on a case-by-case basis. For those cases in which information is available the approach to assess whether or not it is "toxic" under CEPA is similar to that for single "threshold" or "non-threshold toxicants". See Health Canada [17] for an explanation of "toxic", "threshold" and "non-threshold" as defined by CEPA.

Assessing whether simple mixtures are toxic under CEPA can be based on the effects of either some, or all of the components present in the mixture. For those cases in which the components in the simple mixture have similar effects due to similar modes of action, and there is little indication for interaction between components, effects are generally considered to be additive.

One approach for assessing "threshold toxicants" involves determining the total daily intake of the mixture as toxic equivalents (summing of the concentrations of individual compounds multiplied by the potency of that substance relative to that of the reference (generally most potent) substance). This composite measure is then compared to a Tolerable Daily Intake (TDI) for the reference substance, derived as for "threshold toxicants". A hazard index (HI) approach is used for simple mixtures where the components are classified as unlikely to be carcinogenic, to unclassifiable with respect to carcinogenicity in humans and for which the mechanisms of toxicity for the critical effect are similar. The HI is derived in a manner similar to that previously mentioned but substitutes 1/TDI for the relative potency factor. If the numerical value of the hazard index exceeds one, the simple mixture is considered to be toxic under CEPA. In cases where the simple mixture contains a high proportion of substances classified as carcinogenic or probably carcinogenic to humans, or as a human germ cell mutagen or probable mutagen, the mixture as a whole may be considered toxic under CEPA. Such a determination is based on consideration of factors such as the extent of characterization of the chemical composition and toxicological effects of the simple mixture and the proportion of the total mixture that is composed of components classified as (probable) carcinogens/mutagens. For simple mixtures considered to be toxic owing to the classification of a major proportion of components as carcinogens/mutagens, where possible, the estimated daily intake of the components by the general population or concentrations in relevant environmental media are compared to quantitative estimates of carcinogenic or mutagenic potency (Exposure/Potency Index or EPI) to characterize risk and provide guidance in establishing priorities for further action following assessment of toxicity under the Act [17].

The USA Environmental Protection Agency (USEPA) has led the charge in the analysis of effects of mixtures in the general population (non-occupational exposure) [1]. This document has been recently reviewed and updated [3]. The method of assessment for a chemical mixture ultimately depends on the quality of data available to determine if the mixture will be assessed as a whole or as components. For any chemical mixture, all possible paths to the final summary should be examined as a relevant assessment method (See Figure 2-1 in [3]). The USEPA stresses the importance not only of the health effects and interactions information available for assessment but the quality of the exposure data. Some of the essentials required to quantify the exposure to chemical mixtures include: concentration of the mixture at the point of contact; duration and frequency of exposure; accuracy and reliability of the measurement techniques used; determination of whether all components have been identified; bioavailability of the mixture for the medium and route of exposure. The USEPA is currently working on developing cumulative risk assessment guidelines. The external review draft of the framework for these guidelines is available [18]. The EPA defines cumulative risk as the combined risks from aggregate exposures to multiple agents or stressors.

The European Union (EU) recently announced an "European Environment and Health Strategy" [19]. Part of this strategy will be science-based and will examine the interactions of pollutants in the environment and the impact on public health. "Traditionally, environmental assessments and policy action have focused on single pollutants in single environmental compartments, such as air, water or soil. There is, however, a strong need to look into how different pollutants react together. We need to better understand how pollutants move in the environment and how we come in contact with them - through air, water, food, and consumer products. We also need to understand how the human body reacts, over a period of time, to the continuous exposure to different pollutants, interacting between each other, often at a low level." [19]

Current and emerging issues

A number of problems continue to plague the accurate assessment of chemical mixtures. While many of the individual components of a mixture may be adequately documented, there is usually a significant lack of toxicity data on the complete mixture. In addition complex chemical interactions within the mixture may result in unforeseen by-products that have an inherent, also unforeseen, toxicity. The lack of adequate exposure information also complicates the assessment (poor dosimetry). Finally, another factor that is not usually discussed and is highly relevant is not only the effect of stress on exposure and resultant toxicity, but also of exposure-induced stress. In their study, Friedman and Lawrence [20] discussed the effect of chemical, physical and psychological stress on health status from exposures.

What's on the horizon?

The major problems that keep coming up are the lack of mixtures toxicology data and valid exposure data. The explosion of genomics and proteomics could prove to be a goldmine with respect to large scale testing of complex mixtures at the molecular biology level of investigation. While providing a wealth of information, these techniques are not well developed enough yet to provide rapid assessments. PBPK modelling is also progressing to the point where it is starting to provide invaluable information to scientists, evaluators and regulators investigating the effects of complex chemical mixtures [21-23]. Small, sensitive dosimeters capable of providing accurate and rapid chemical exposure data are being developed that will provide exposure information that is lacking or not representative of exposure at the level of entry into the body. Multimedia exposure assessment strategies have developed significantly over the last 10 years and coupled with realistic exposure data have rapidly expanded the ability of the toxicologist/evaluator to assess the dose presented to biological systems.

Conclusions

The issue of understanding the effects from exposure to chemical mixtures is not trivial. In many ways this part of toxicology is in its infancy. It also presents significant problems to evaluators/regulators as each chemical mixture can be geographically distinct in the environment (e.g. air pollutants). Different government and non-government bodies have developed detailed strategies to assess what levels may be considered safe but there are several limitations to these strategies. Many do not account for interactions either at a chemical or biological level, or are only effective if there is sufficient toxicological and exposure information on the whole mixture. If this information is not present, the task of assessing the mixture becomes filled with uncertainty. Another problem is that it is assumed that the exposure and subsequent health effects are from exposure to the native form of the mixture and not to the mixture after it has been physically altered (e.g. combustion) and mixed with other compounds or mixtures. There is significant effort being applied to the assessment of exposures to mixtures whether the exposure is occupational or environmental. The information, guidelines and regulations coming from these efforts can act as a guide to

assist in developing methodologies to assess risk to troops in different chemical environments. Developing and validating these models and algorithms would be a significant undertaking.

The rapid expansion of techniques being exploited by toxicologists will provide data in the future that will greatly assist in the assessment of exposures to mixtures. While some of these techniques may not speed up the assessment process, they will bring a volume of data to the assessment arena that has not been seen before. The examination of whole complex mixtures will benefit from the advancement of genomics, proteomics, and PBPK modelling but the process of assessment itself will become more complex and difficult.

References

1. United States Environmental Protection Agency (1986). Guidelines for the Health Risk Assessment of Chemical Mixtures. (EPA/630/R-98/002). United States Environmental Protection Agency.
2. Agency for Toxic Substances and Disease Registry (2001). Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures - Draft for Public Comment. US Dept of Health and Human Services.
3. United States Environmental Protection Agency (2000). Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. (EPA/630/R-00/002). United States Environmental Protection Agency.
4. Calabrese, E.J. and Baldwin, L.A. (2003). Toxicology rethinks its central belief. *Nature*, 421, 691-692.
5. Calabrese, E.J. and Baldwin, L.A. (2002). Applications of hormesis in toxicology, risk assessment and chemotherapeutics. *Trends Pharmacol. Sci.*, 23 (7), 331-337.
6. Jayjock, M.A. and Lewis, P.G. (2002). Implications of hormesis for industrial hygiene. *Hum. Exp. Toxicol.*, 21 (7), 385-389.
7. Workplace Hazard Material Information System (WHMIS). (Online) Health Canada <http://www.hc-sc.gc.ca/hecs-sesc/whmis/index.htm> (21 Nov. 2003).
8. Occupational Safety and Health Standards. (Online) Occupational Safety and Health Administration <http://www.osha.gov/> (2004).
9. Department of Health and Human Services (2002). Exposure Assessment Methods: Research Needs and Priorities. (DHHS (NIOSH) Publication No. 2002-126). Department of Health and Human Services.
10. Scientific Committee on Occupational Exposure Limits (SCOEL). (Online) European Commission http://europe.eu.int/comm/employment_social/health_safety/areas/oels_en.htm (2004).
11. National Industrial Chemicals Notification and Assessment Scheme (NICNAS). (Online) Department of Health and Ageing, A.G. <http://www.nicnas.gov.au/> (4 May 2004).
12. National Occupational Health and Safety Commission (NOHSC). (Online) National Occupational Health and Safety Commission, A.G. <http://www.nohsc.gov.au/> (2004).
13. The National Academies. (Online) National Academy of Sciences <http://www.nationalacademies.org/> (20 Aug. 2002).
14. American Conference of Governmental Industrial Hygienists (2003). 2003 TLVs and BEIs. p 224, Cincinnati, Ohio: ACGIH, Inc.
15. The Globally Harmonized System of Classification and Labelling of Chemicals. (Online) United Nations Economic Commission for Europe <http://www.unece.org/trans/danger/publi/ghs/officialtext.html> (14 June 2004).

16. Organization for Economic Co-operation and Development (2001). Harmonised Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures. OECD Series on Testing and Assessment Number 33. Paris, France: Organization for Economic Co-operation and Development.
17. Health Canada (1994). Human Health Risk Assessment for Priority Substances. Ottawa, Canada: Canada Communication Group - Publishing.
18. United States Environmental Protection Agency (2002). Framework for Cumulative Risk Assessment - External Review DRAFT. (EPA/630/P-02/001A). United States Environmental Protection Agency.
19. European Commission (2003). Environment and Health; the European Commission Launches a Strategy to Reduce Diseases Linked to Environmental Factors. (IP/03/823). Brussels, Belgium: European Commission.
20. Friedman, E.M. and Lawrence, D.A. (2002). Environmental stress mediates changes in neuroimmunological interactions. *Toxicol. Sci.*, 67 (1), 4-10.
21. Conolly, R.B. (2001). Biologically motivated quantitative models and the mixture toxicity problem. *Toxicol. Sci.*, 63 (1), 1-2.
22. Haddad, S., Beliveau, M., Tardif, R. and Krishnan, K. (2001). A PBPK modeling-based approach to account for interactions in the health risk assessment of chemical mixtures. *Toxicol. Sci.*, 63 (1), 125-131.
23. Haddad, S., Charest-Tardif, G., Tardif, R. and Krishnan, K. (2000). Validation of a physiological modeling framework for simulating the toxicokinetics of chemicals in mixtures. *Toxicol. Appl. Pharmacol.*, 167 (3), 199-209.

Annexes

Annex 1: Links to relevant organizations

Agency for Toxic Substances and Disease Registry (ATSDR); United States of America:
<http://www.atsdr.cdc.gov/>

American Conference of Governmental and Industrial Hygienists (ACGIH); United States of America: <http://www.acgih.org/>

Canadian Environmental Protection Act (CEPA); Canada:
<http://www.ec.gc.ca/CEPARRegistry/default.cfm>

Environmental Protection Agency (USEPA); United States of America: <http://www.epa.gov/>

European Environment and Health Strategy (EEHS); European Union:
http://europa.eu.int/comm/press_room/presspacks/health/pp_health_en.htm

Globally Harmonized System of Classification and Labelling of Chemicals (GHS); United Nations Economic Commission for Europe:
<http://www.unece.org/trans/danger/publi/ghs/officialtext.html>

National Academies of Science; United States of America: <http://www.nationalacademies.org/>

National Industrial Chemicals Notification and Assessment Scheme (NICNAS); Australia:
<http://www.nicnas.gov.au/>

National Institutes for Occupational Safety and Health (NIOSH); United States of America:
<http://www.cdc.gov/niosh/homepage.html>

National Occupational Health and Safety Commission (NOHSC); Australia:
<http://www.nohsc.gov.au/>

Occupational Safety and Health Administration (OSHA); United States of America:
<http://www.osha.gov/>

Organisation for Economic Co-operation and Development (OECD):
<http://www.oecd.org/home/>

Scientific Committee on Occupational Exposure Limits (SCOEL); European Union:
http://europa.eu.int/comm/employment_social/health_safety/areas/oels_en.htm

Workplace Hazard Material Information System (WHMIS); Canada:
<http://www.hc-sc.gc.ca/hecs-sesc/whmis/index.htm>

List of acronyms

ACGIH	American Conference for Governmental Industrial Hygienists
ATSDR	Agency for Toxic Substances and Disease Registry
C/T	Ratio of Exposure Concentration to TLV
CEPA	Canadian Environmental Protection Act
CFR	Code of Federal Regulations
CPR	Controlled Products Regulations
EPI	Exposure/Potency Index
GHS	Globally Harmonised System
HI	Hazard Index
ISS	Integral Search System
MRL	Minimal Risk Levels
NIOSH	National Institutes for Occupational Safety and Health
NOHSC	National Occupational Health and Safety Commission
OECD	Organization for Economic Cooperation and Development
OEL	Occupational Exposure Limits
OSHA	Occupational Safety and Health Administration
PBPK	Physiologically-Based Pharmacokinetic Modelling
PBPK/PD	Physiologically-Based Pharmacokinetic/Pharmacodynamic
PEL	Permissible Exposure Limit
SCOEL	Scientific Committee on Occupational Exposure Limits
STEL	Short-Term / Excursion Limits
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TDI	Tolerable Daily Intake

TLV	Threshold Limit Value
TTD	Target-organ Toxicity Dose
TWA	Time Weighted Averages
UNECE	United Nations Economic Commission for Europe
USEPA	USA Environmental Protection Agency
WHMIS	Workplace Hazardous Materials Information System
WOE	Weight-of-Evidence Modification

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The science of risk assessment revolves broadly around hazard identification (toxicity) and exposure assessment information. While exposure to environmental hazards most often occurs with complex chemical mixtures, the majority of existing toxicity data is for single compounds or simple mixtures, thus presenting problems to the risk assessor. Several approaches to assess mixtures have been developed (e.g. Hazard Index; Target-organ Toxicity Dose; Weight-of Evidence; Toxic Equivalence), each of which have their limitations, primarily with respect to the prediction of potentially unforeseen interactions between the mixture constituents which may affect their resultant toxic outcome. Recent advances in disciplines such as genomics, proteomics, metabonomics and physiologically-based pharmacokinetic modeling should assist in the hazard assessment of complex chemical mixtures. However, the process of regulatory assessment of these types of exposures will remain both complex and difficult.

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